09/764,050

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Term: L2 and immobiliz$2

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DATE: Thursday, August 22, 2002 Printable Copy Create Case

Set Name side by side	Query	Hit Count	Set Name result set
DB=USPT,JPAB,EPAB,DWPI; PLUR=YES; OP=ADJ			
L3	L2 and immobiliz$2	1	L3
L2	L1 and nucleic acid	11	L2
L1	(dried or drying) near5 fluorescence near5 detect$	20	L1

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L3: Entry 1 of 1 File: USPT Aug 21, 2001

DOCUMENT-IDENTIFIER: US 6277628 B1
TITLE: Linear microarrays

Brief Summary Text (8):
The present invention provides a hybridization composition for detecting the levels of a plurality of biomolecular probes in a sample. The composition comprises (a) a capillary-like casing; and (b) a substrate immobilized in said casing. The substrate's surface contains a plurality of regions arranged in a defined manner with respect to the length of said casing and each of the regions has one or more different immobilized target. Additionally, the substrate's surface is in close proximity with the inner surface of said casing so as to minimize the ratio of liquid volume contained within said casing to the substrate's surface area. The linear density of the plurality of defined regions is greater than 1.times.10.sup.3 /cm, preferably is greater than 1.times.10.sup.3 /cm, and more preferably is greater than 1.times.10.sup.6 /cm.

Brief Summary Text (10):
The present invention also provides a method for detecting the levels of a plurality of biomolecular probes in a sample. The method comprises (i) contacting the sample comprising the plurality of biomolecular probes with a hybridization composition comprising (a) a capillary-like casing and (b) a substrate immobilized in said casing under conditions effective to form hybridization complexes between biomolecular probes and immobilized targets; and (ii) detecting the hybridization complexes.

Detailed Description Text (3):
The invention is a composition comprising one or more capillary-like casings with one or more input openings and one or more output openings and a substrate immobilized in each of said capillary-like casings. A key feature of the invention is that the substrate's surface is in close proximity with the inner surface of the capillary casing so as to minimize the ratio of liquid volume contained within said casing to the substrate's surface area. In this manner the volume for sample delivery and hybridization reactions is minimized. Typically, for two-dimensional microarrays the ratio of sample volume to surface area is about 1.times.10-.sup.5 m. In contrast, the present invention provides for ratios of sample volume to hybridization surface area less than 1.times.10.sup.-5 m, preferably less than 1.times.10-.sup.7 m, and more preferably less than 1.times.10.sup.-7 m. The ratios can be varied by changing, for example, the inner diameter of the capillary-like casing or the surface area of the substrate.

Detailed Description Text (4):
One preferred embodiment of the present invention is illustrated in FIG. 1. In this embodiment, the capillary-like casing is a capillary tube 2 and the substrate comprises a plurality of beads, such as bead 4. Each bead has at least one type of immobilized target, such as immobilized target 6, on its surface. A key feature of the invention is that each bead is localized in defined locations along the length of the casing, such that the hybridization composition is defined by having a linear density of different polynucleotide sequences of at least 1/cm, preferably at least 1.times.10.sup.3 /cm, more preferably greater than 1.times.10.sup.6 /cm. Additionally, the inner diameter of the capillary and the diameter of the bead are selected to be substantially similar. This means the diameter of the bead is more than 90%, preferable more than 95%, and more preferably more than 98%, the length of the inner diameter of the capillary-like casing. Samples, prehybridization buffers, hybridization buffers, and washes flow into the hybridization composition through either first 8 or second 10 open ends and flow out, preferably, through the opposite

end.

Detailed Description Text (5):
A second preferred embodiment is illustrated in FIG. 2. In this second embodiment, the capillary-like casing is a capillary tube 12 and the substrate comprises a rod 14 coextensive with the capillary tube. The rod may comprise bands, such as band 16 containing at least one type of immobilized target, such as target 18. Band regions containing different immobilized targets may alternate with bands lacking a target, such as unmodified band 20. Again, the hybridization composition is defined by having a linear density of different polynucleotide sequences of at least 1/cm, preferably at least 1.times.10.sup.3 /cm, more preferably greater than 1.times.10.sup.6 /cm. Additionally, the inner radius of the capillary tube and the outer radius of the rod ;re substantially similar. Samples, prehybridization buffers, hybridization buffers, or washes flow into the hybridization composition through either first 22 or second 24 open ends and flow out, preferably, through the opposite end. Alternatively, the substrate may comprise alternating agarose plugs modified by at least one target and unmodified agarose plugs.

Detailed Description Text (6):
A third embodiment is illustrated in FIG. 3. In this instance, the capillary-like casing is a capillary tube 30 and the substrate comprises a rod 32 coextensive with the capillary tube. In this case, however, instead of having defined regions, such as defined region 34, running perpendicular to the length of the casing, the discrete regions run in parallel to the length of the casing. Again, each defined region may contain one or more immobilized targets, such as target 36. Alternatively, the substrate may comprise a plurality of rods containing different polynucleotide sequences in defined regions along the length of the casing.

Detailed Description Text (7):
FIG. 4 illustrates how the present invention is implemented in a format where a plurality of hybridization compositions are employed at the same time. As shown in FIG. 4, three hybridization compositions, such as hybridization composition 40, is connected to one or more neighboring hybridization compositions to form a two-dimensional arrangement 42. Alternatively, the hybridization compositions can be in a three-dimensional arrangement. Connections, such as valve 44, between neighboring hybridization compositions 40 and 46 can be in an open or closed position. When connections are in a closed position, different hybridizing compositions can be subjected to different samples and/or hybridizing conditions. The dimensions of the capillary-like casing, the substrate, the defined regions or the immobilized targets are not drawn to scale in FIGS. 1 through 4.

Detailed Description Text (12):
Immobilized on a plurality of defined regions of the substrate's surface, are localized multiple copies of one or more polynucleotide sequences, preferably copies of a single polynucleotide sequence. A polynucleotide refers to a chain of nucleotides. Preferably, the chain has from 5 to 10,000 nucleotides, more preferably from 15 to 3,500 nucleotides.

Detailed Description Text (13):
The plurality of defined regions on the substrate can be arranged in a variety of formats. For example, the regions may be arranged perpendicular or in parallel to the length of the casing. These immobilized copies of a polynucleotide sequence are suitable for use as a target polynucleotide in hybridization experiments. Furthermore, the probes do not have to be directly bound to the substrate, but rather can be bound to the substrate through a linker group. The linker groups may typically vary from about 6 to 50 atoms long. Preferred linker groups include ethylene glycol oligomers, diamines, diacids and the like. Reactive groups on the substrate surface react with one of the terminal portions of the linker to bind the linker to the substrate. The other terminal portion of the linker is then functionalized for binding the polynucleotides.

Detailed Description Text (14):
To prepare beads coated with immobilized polynucleotide sequences, beads are immersed in a solution containing the desired polynucleotide sequence and then immobilized on the beads by covalent or noncovalent means. Alternatively, when the polynucleotides

are immobilized on rods, a given polynucleotide can be spotted at defined regions of the rod. Typical dispensers include a micropipette delivering solution to the substrate with a robotic system to control the position of the micropipette with respect to the substrate. There can be a multiplicity of dispensers so that reagents can be delivered to the reaction regions simultaneously. In one embodiment, a microarray is formed by using ink-jet technology based on the piezoelectric effect, whereby a narrow tube containing a liquid of interest, such as oligonucleotide synthesis reagents, is encircled by an adapter. An electric charge sent across the adapter causes the adapter to expand at a different rate than the tube and forces a small drop of liquid onto a substrate (Baldeschweiler et al. PCT publication WO95/251116).

Detailed Description Text (15):
Samples may be any sample containing polynucleotides (polynucleotide probes) of interest and obtained from any bodily fluid (blood, urine, saliva, phlegm, gastric juices, etc.), cultured cells, biopsies, or other tissue preparations. DNA or RNA can be isolated from the sample according to any of a number of methods well known to those of skill in the art. For example, methods of purification of nucleic acids are described in Laboratory Techniques in Biochemistry and Molecular Biology: Hybridization With Nucleic Acid Probes. Part I. Theory and Nucleic Acid Preparation, P. Tijssen, ed. Elsevier (1993). In. a preferred embodiment, total RNA is isolated using the TRIzol total RNA isolation reagent (Life Technologies, Inc., Rockville, Md.) and RNA is isolated using oligo d(T) column chromatography or glass beads. After hybridization and processing, the hybridization signals obtained should reflect accurately the amounts of control target polynucleotide added to the sample.

Detailed Description Text (17):
Labeling can be carried out during an amplification reaction, such as polymerase chain reaction and in vitro or in vivo transcription reactions. Alternatively, the labeling moiety can be incorporated after hybridization once a probe-target complex his formed. In one preferred embodiment, biotin is first incorporated during an amplification step as described above. After the hybridization reaction, unbound nucleic acids are rinsed away so that the only biotin remaining bound to the substrate is that attached to target polynucleotides that are hybridized to the polynucleotide probes. Then, an avidin-conjugated fluorophore, such as avidin-phycoerythrin, that binds with high affinity to biotin is add

Detailed Description Text (20):
Hybridization reactions can be performed in absolute or differential hybridization formats. In the absolute hybridization format, polynucleotide probes from one sample are hybridized to the target sequences and signals detected after hybridization complex formation correlate to polynucleotide probe levels in a sample. In the differential hybridization format, the differential expression of a set of genes in two biological samples is analyzed. For differential hybridization, polynucleotide probes from both biological samples are prepared and labeled with different labeling moieties. A mixture of the two labeled polynucleotide probes is added to a microarray. After hybridization and washing, the microarray is examined under conditions in which the emissions from the two different labels are individually detectable. Probes in the microarray that are hybridized to substantially equal numbers of target polynucleotides derived from both biological samples give a distinct combined fluorescence (Shalon et al. PCT publication WO95/35505). In a preferred embodiment, the labels are fluorescent labels with distinguishable emission spectra, such as Cy3/Cy5 fluorophores (Amersham Pharmacia Biotech, Piscataway, N.J.). After hybridization, the microarray is washed to remove nonhybridized nucleic acids and complex formation between the hybridizable array elements and the target polynucleotides is detected.

Detailed Description Text (32):
The array, attached to its glass slide, was immersed in an electrophoresis chamber filled with 1M MOPS with the capillary parallel to the electric field. One microliter of a 400 uM solution of a Cy5-5'-labeled 59mer was added to the end of an agarose plugged capillary. The sequence of this second polynucleotide was complementary to the agarose-immobilized polynucleotide.

Detailed Description Text (33):

A potential of 40 volts was applied to the chamber for two hours. Over this time the Cy5-labeled polynculeotide was observed to removed from the chamber and scanned with a confocal fluorescence microscope using a Cy3 and Cy5 detection system. The Cy5-labeled polynucleotide was found to localize only in regions where complementary Cy3-labeled polynucleotide was immobilized.

Detailed Description Text (35):
100 ul of a 4 uM Cy5-5'labeled 59mer in 5.times.SSC,0.1%SDS was used to fill a bead capillary array. The array was then incubated for 2 hours at 60.degree. C. It was then washed in succession with 200 ul 5.times.SSC, 0.1% SDS, 200 ul 0.5.times.SSC, 0.1%SDS, and 200 ul 0.1%SDS. The array was then dried under vacuum and scanned with a confocal fluorescence microscope using a Cy3 and Cy5 detection system. The Cy5-labeled polynucleotide was found to localize in regions where complementary Cy3-polynucleotide derivatized igarose was present.

CLAIMS:

1. A device for detecting a plurality of biomolecular probes in a sample, said device comprising a vessel comprising:

(a) a casing having an inner surface and input and output openings,

(b) a nonporous substrate having an outer surface and contained within said casing,

(c) a liquid sample comprising biomolecular probes and contained within said casing,

wherein the substrate outer surface contains a plurality of regions arranged in a defined manner and each of said regions has a different one of a plurality of immobilized specific binding targets which specifically bind a corresponding one of the plurality of the biomolecular probes, and said substrate outer surface is in close proximity with the inner surface of said casina so as to minimize the ratio of the liquid sample volume contained within said casing to the substrate outer surface area, yet permit flow of the liquid sample from the input opening, between the substrate outer surface and the casing inner surface, and to the output opening.

17. The device of claim 1, wherein the immobilized binding targets and corresponding biomolecular probes comprise polynucleotides which specifically bind by hybridization.

18. The device of claim 1, wherein the ratio of the liquid sample volume contained within the casing to the substrate outer surface area is less than about 1.times.10.sup.-5 m, and the immobilized binding targets and corresponding biomolecular probes comprise polynucleotides which specifically bind by hybridization.

19. The device of claim 1, wherein the ratio of the liquid sample volume contained within the casing to the substrate outer surface area is less than about 1.times.10.sup.-7 m, and the immobilized binding targets and corresponding biomolecular probes comprise polynucleotides which specifically bind by hybridization.

20. The device of claim 1, wherein the ratio of the liquid sample volume contained within the casing to the substrate outer surface area is less than about 1.times.10.sup.-5 m, the substrate is elongate and has a length and the regions are arranged with a linear density of greater than 1.times.10.sup.3 regions per centimeter of the length, and the immobilized binding targets and corresponding biomolecular probes comprise polynucleotides which specifically bind by hybridization.

21. The device of claim 1, wherein the ratio of the liquid sample volume contained within the casing to the substrate outer surface area is less than about 1.times.10.sup.-7 m, the substrate is elongate and has a length and the regions are arranged with a linear density of greater than 1.times.10.sup.3 regions per centimeter of the length, and the immobilized binding targets and corresponding biomolecular probes comprise polynucleotides which specifically bind by hybridization.

22. The device of claim 1, wherein the ratio of the liquid sample volume contained within the casing to the substrate outer surface area is less than about 1.times.10.sup.5 m, the immobilized binding targets and corresponding biomolecular probes comprise polynucleotides which specifically bind by hybridization, and the substrate is made of glass, optionally surface coated with a polymer to promote immobilization of the binding targets.

23. The device of claim 1, wherein the ratio of the liquid sample volume contained within the casing to the substrate outer surface area is less than about 1.times.10.sup.-7 m, the immobilized binding targets and corresponding biomolecular probes comprise polynucleotides which specifically bind by hybridization, and the substrate is made of glass, optionally surface coated with a polymer to promote immobilization of the binding targets.

24. The device of claim 1, wherein the ratio of the liquid sample volume contained within the casing to the substrate outer surface area is less than about 1.times.10.sup.-5 m, the substrate is elongate and has a length and the regions are arranged with a linear density of greater than 1.times.10.sup.3 regions per centimeter of the length, the immobilized binding targets and corresponding biomolecular probes comprise polynucleotides which specifically bind by hybridization and the substrate is made of glass, optionally surface coated with a polymer to promote immobilization of the binding targets.

25. The device of claim 1, wherein the ratio of the liquid sample volume contained within the casing to the substrate outer surface area is less than about 1.times.10.sup.-7 m, the substrate is elongate and has a length and the regions are arranged with a linear density of greater than 1.times.10.sup.3 regions per centimeter of the length, the immobilized binding targets and corresponding biomolecular probes comprise polynucleotides which specifically bind by hybridization and the substrate is made of glass, optionally surface coated with a polymer to promote immobilization of the binding targets.

26. A method for detecting biomolecular probes in a sample, said method comprising the step of using a device according to claim 1 to detect specific binding of the biomolecular probes to the immobilized targets.

27. A method for detecting biomolecular probes in a sample, said method comprising the step of using a device according to claim 2 to detect specific binding of the biomolecular probes to the immobilized targets.

28. A method for detecting biomolecular probes in a sample, said method comprising the step of using a device according to claim 3 to detect specific binding of the biomolecular probes to the immobilized targets.

29. A method for detecting biomolecular probes in a sample, said method comprising the step of using a device according to claim 4 to detect specific binding of the biomolecular probes to the immobilized targets.

30. A method for detecting biomolecular probes in a sample, said method comprising the step of using a device according to claim 5 to detect specific binding of the biomolecular probes to the immobilized targets.

31. A method for detecting biomolecular probes in a sample, said method comprising the step of using a device according to claim 6 to detect specific binding of the biomolecular probes to the immobilized targets.

32. A method for detecting biomolecular probes in a sample, said method comprising the step of using a device according to claim 16 to detect specific binding of the biomolecular probes to the immobilized targets.

33. A method for detecting biomolecular probes in a sample, said method comprising the step of using a device according to claim 17 to detect specific binding of the biomolecular probes to the immobilized targets.

34. A method for detecting biomolecular probes in a sample, said method comprising the

step of using a device according to claim 18 to detect specific binding of the biomolecular probes to the immobilized targets.

35. A method for detecting biomolecular probes in a sample, said method comprising the step of using a device according to claim 19 to detect specific binding of the biomolecular probes to the immobilized targets.

36. A method for detecting biomolecular probes in a sample, said method comprising the step of using a device according to claim 20 to detect specific binding of the biomolecular probes to the immobilized targets.

37. A method for detecting biomolecular probes in a sample, said method comprising the step of using a device according to claim 21 to detect specific binding of the biomolecular probes to the immobilized targets.

38. A method for detecting biomolecular probes in a sample, said method comprising the step of using a device according to claim 22 to detect specific binding of the biomolecular probes to the immobilized targets.

39. A method for detecting biomolecular probes in a sample, said method comprising the step of using a device according to claim 23 to detect specific binding of the biomolecular probes to the immobilized targets.

40. A method for detecting biomolecular probes in a sample, said method comprising the step of using a device according to claim 24 to detect specific binding of the biomolecular probes to the immobilized targets.

41. A method for detecting biomolecular probes in a sample, said method comprising the step of using a device according to claim 25 to detect specific binding of the biomolecular probes to the immobilized targets.

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L7	resin near5 hybridiz$	42	L7
L6	(((resin near5 hybridization)and nucleic acid) and immobiliz$) AND @pd>20020822	0	L6
L5	(((resin near5 hybridization)and nucleic acid) and immobiliz$)	4	L5
L4	(((resin near5 hybridization)and nucleic acid) and immobiliz$)	4	L4
L3	JP404330300.pn.	0	L3
L2	JP404330300	0	L2
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☑ 1. 6348348 . 07 Apr 99; 19 Feb 02. Human hairless gene and protein. Thompson; Catherine C.. 435/320.1; 435/6 435/7.1 530/350 536/23.1. C12N015/00.

☐ 2. 5773213 . 06 Jun 94; 30 Jun 98. Method for conducting sequential nucleic acid hybridization steps. Gullans; Steven R., et al. 435/6; 435/91.1 435/91.2 536/24.32 536/24.33. C07H021/02 C07H021/04 C12P019/34 C12Q001/68.

☐ 3. 5753439 . 19 May 95; 19 May 98. Nucleic acid detection methods. Smith; Cassandra L., et al. 435/6; 435/5 435/91.2 536/24.3 536/24.32 536/24.33. C12Q001/68 C12Q001/70 C12P019/34 C07H021/04.

☐ 4. JP 04330300 A . 25 Apr 91. 18 Nov 92. METHOD FOR IMMOBILIZING NUCLEIC ACID TO MEMBRANE. MIYAKOSHI, TERUICHI, et al. 435/6. C12Q001/68;.

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Term	Documents
RESIN.DWPI,EPAB,JPAB,USPT.	1443529
RESINS.DWPI,EPAB,JPAB,USPT.	283592
HYBRIDIZATION.DWPI,EPAB,JPAB,USPT.	34991
HYBRIDISATION.DWPI,EPAB,JPAB,USPT.	5418
HYBRIDISATIONS.DWPI,EPAB,JPAB,USPT.	245
HYBRIDIZATIONS.DWPI,EPAB,JPAB,USPT.	5002
NUCLEIC.DWPI,EPAB,JPAB,USPT.	80127
NUCLEICS.DWPI,EPAB,JPAB,USPT.	9
ACID.DWPI,EPAB,JPAB,USPT.	1638037
ACIDS.DWPI,EPAB,JPAB,USPT.	479501
IMMOBILIZ$	0
((((RESIN NEAR5 HYBRIDIZATION)AND NUCLEIC ACID) AND IMMOBILIZ$)).USPT,JPAB,EPAB,DWPI.	4

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Previous Page Next Page

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L5: Entry 1 of 4 File: USPT Feb 19, 2002

DOCUMENT-IDENTIFIER: US 6348348 B1
TITLE: Human hairless gene and protein

Drawing Description Text (36):
A screening method may comprise incubating Hr protein (or fragment thereof) with a candidate chemical agent and thyroid hormone receptor (or fragment thereof); and determining the amount of the thyroid hormone receptor (or fragment thereof) which is bound to Hr protein (or fragment thereof), the desired chemical agent being one which increases or decreases binding. Preferably, at least one of the Hr protein (or fragment thereof) and the thyroid hormone receptor (or fragment thereof) is immobilized to a solid substrate to facilitate separation of bound from unbound complexes.

Drawing Description Text (44):
According to another embodiment of the invention, candidate chemical agents regulating the binding between Hr protein and thyroid hormone receptor may be identified. Hr protein can be attached to an insoluble polymeric support such as acrylamide, agarose, cellulose, or plastics, or other supports such as glass. A candidate chemical agent is incubated with the immobilized Hr protein in the presence of thyroid hormone receptor. Alternatively, thyroid hormone receptor can be immobilized on a solid support and a candidate chemical agent can be incubated with the immobilized thyroid hormone receptor in the presence of Hr protein. After incubation, non-binding components can be washed away, leaving thyroid hormone receptor bound to Hr protein/solid support or Hr protein bound to thyroid hormone receptor/solid support, respectively. Washing may be facilitated by forming the solid support into a bilious strip, a well of a 96-well plate, a bead, a chromatography column, or a porous membrane. Solution transfer may be accomplished by fluid channels, magnetic particles, or robotics.

Drawing Description Text (68):
Further, the polynucleotide, polypeptide, and specific binding molecule may be optionally attached to a solid substrate (e.g., glass or silanized slide, magnetic bead, microtiter plate, nitrocellulose, nylon, resin bead). Such reagent would allow capture of a molecule in solution by a specific interaction between the cognate molecules and immobilization of the solution molecule on the solid substrate. See, for example, U.S. Pat. Nos. 5,143,854; 5,639,603; 5789,162; and 5,789,172. Monitoring Hairless expression is facilitated by using biochips or microarrays. See, for example, U.S. Pat. Nos. 5,445,934; 5,510,270; 5,545,531; 5,677,195; and a special supplement (Nat. Genet., 21, 1-60,1999).

Drawing Description Text (69):
Nucleotide and amino acid sequences may be synthesized in situ on the substrate by solid phase chemistry or photolithography. In situ synthesis attaches the nucleotides or amino acids directly to the substrate. Alternatively, the polynucleotide, polypeptide, or specific binding molecule may be attached by interaction of a specific binding pair (e.g., antibody-digoxygenin/hapten/peptide, biotin-avidin/streptavidin, GST-glutathione, MBP-maltose, polyhistidine-nickel, protein A/G-immunoglobulin); crosslinking may be used if covalent attachment to the substrate is desired. Glutaraldehyde is a covalent bifunctional crosslinker suitable for immobilization on a substrate, but a photoactivatable, reversible crosslinker is preferred to identify and isolate molecules interacting in a complex (e.g., a thiol linkage that may be reduced).

Drawing Description Text (70):
Hybridization may take place in solution or on a solid substrate. If either the Hairless polynucleotide or probe that undergoes hybridization is attached to a solid substrate (e.g., glass or silanized slide, magnetic bead, microtiter plate,

nitrocellulose, nylon, resin bead), hybridization will result in capture of the unattached species.

Detailed Description Text (14):
To construct pLexA-hr, a 2.2 Kb Hind III fragment corresponding to amino acids 575-1215 of hr (FIG. 1) was isolated, the ends filled-in with Klenow large fragment, Bam HI linkers ligated, and then cloned into the Bam HI site of pLexA (Hollenberg et al., Mol. Cell Biol., 15, 3813-3822, 1995). The resulting plasmid was transformed into yeast strain L40 (Hollenberg et al., ibid.). The resulting strain was used to screen a human brain cDNA library constructed as a fusion with the activation domain of VP16. DNA was isolated from HIS+, lacZ+ colonies (Robzyk and Kassir, Nucleic Acids Res., 20, 3790, 1992), propagated in E. coli, purified and sequenced. Cells were tested for .beta.-galactosidase activity as described (Reynolds and Lundblad, in Short Protocols in Molecular Biology, Ausubel et al., eds., John Wiley, New York, p. 13-27, 1992). To test the hormone dependence of interaction, TRIAC (Sigma) was added to the media and assay buffer (final concentration 10.sup.-6 M).

Detailed Description Text (18):
To confirm the direct interaction between hr and TR, a far western assay was used. hr (amino acids 575-1215) was expressed in bacteria as a fusion protein with either glutathione S transferase (GST) or TrpE. Extracts from bacteria expressing hr fusion proteins were separated by SDS-polyacrylamide gel electrophoresis and transferred to nitrocellulose: TrpE-Srg1 (negative control), TrpE-hr, GST only, and GST-hr. The immobilized, renatured proteins were incubated with .sup.35 S-TR.alpha.1. TR.alpha.1 detected a protein the size of the hr fusion proteins, which was recognized by hr-specific antisera. Therefore, hr interacts specifically with TR.alpha.1. These data also show that no other factors (for example, other proteins in yeast) are required for this interaction.

Detailed Description Text (47):
The plasmid containing the 3.5 Kb cDNA insert (clone 37MC1) of the human homolog of the mouse hairless gene was nick-translated with biotin-14 dATP (Gibco-BRL), with 20% incorporation as determined by tritium tracer incorporation. Slides with chromosome spreads were made from normal male lymphocytes cultured with BrdU (Bhatt et al., Nucleic Acids Res. 16, 3951-3961, 1988). Fluorescence in situ hybridization was performed as described (Lichter et al., Science 247, 64-69, 1990) with modifications. Probe mix (2.times.SSCP, 50% formamide, 10% dextran sulfate, 20 ng/.mu.l biotinylated probe, and 200 g/.mu.l salmon sperm DNA) was denatured at 70.degree. C. for 5 minutes, quickly chilled on ice, placed on slides and hybridized at 37.degree. C. overnight. Slides were washed in 50% formamide/2.times.SSC at 37.degree. C. for 20 minutes, and two changes of 2.times.SSC at 37.degree. C. for 5 minutes each. Biotinylated probe was detected with FITC-avidin and amplified with biotinylated anti-avidin, using an in situ hybridization kit (Oncor) and manufacturer's instructions.